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PKD2 Cation Channel Is Required for Directional Sperm Movement and Male Fertility

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Summary

Sperm of both mammals and invertebrates move toward specific sites in the female reproductive tract. However, molecular mechanisms for sperm to follow directional cues are unknown. Here, we report genetic analysis of *Drosophila Pkd2* at 33E3 (*Pkd2*, *CG6504*), which encodes a Ca^{2+}-activated, nonselective cation channel homologous to the human *Pkd2* autosomal dominant polycystic kidney disease (ADPKD) gene [1]. The PKD2 family of genes has been implicated in sensory responses through protein localization on primary cilia of epithelia and neurons [2–4]. In renal tubules, cilium-associated PKD2 appears to mediate Ca^{2+} influx in response to fluid flow, and the loss of fluid sensation probably contributes to cyst growth and ADPKD [4]. Sperm tails or flagella are specialized cilia essential for movement. *Drosophila Pkd2* is abundantly associated with the tail and the acrosome-containing head region of mature sperm. Targeted disruption of *Pkd2* results in male sterility without affecting spermatogenesis. The mutant sperm are motile but fail to swim into the storage organs in the female. Rare mutant sperm that reach the storage organs are able to fertilize the egg and produce viable progeny. Our data demonstrate that the *Drosophila* PKD2 cation channel operates in sperm for directional movement inside the female reproductive tract.

Results and Discussion

Drosophila PKD2 protein is most abundantly expressed in the larval and adult testes. The testis tip contains mitotic stem cells that show very little or no PKD2 expression (Figures 1A and 1B). In contrast, all differentiating sperm precursors and the mature sperm contain high levels of PKD2 (Figure 1). PKD2 appears as dotted staining along the entire sperm length, the tail, and the head plus the acrosome region, with no obvious regional differences (Figures 1D–1F). Since the tail (1.9 mm) is substantially longer than the head (9 μm), most PKD2 protein is associated with the tail. This staining pattern is especially obvious in individualized sperm tail double labeled with antibodies for axonemal tubulin and PKD2 (Figure 1F).

By using the targeted knockout protocol [5] that involves homologous recombination between a mutated *Pkd2* donor sequence and the endogenous *Pkd2* locus, we obtained $Pkd2^{ko67}$, $Pkd2^{ko42}$, and $Pkd2^{ko2}$ alleles (see figure and details in Supplemental Data). All alleles produce no detectable levels of wild-type size PKD2 (Figures 1C and 2A) and are homozygous viable and phenotypically strong loss-of-function mutations (Figure 2B). Homozygous males of these alleles show normal testis development but greatly reduced fertility. The average number of F1 progeny produced by one mated wild-type female in 48 hr is reduced from 60 for a wild-type male parent to 3 for a $PKD2^{ko67}$ male parent (Figure 2B). One copy of wild-type *Pkd2* cDNA transgene restores the fertility to approximately 50% of the wild-type level (Figure 2B), demonstrating that inactivation of *Pkd2* is the underlying cause of male fertility reduction.

To investigate this further, we used a sperm tail-associated GFP reporter (*dj-GFP*) [6] to quantify sperm production by examining the seminal vesicles (sv) where mature sperm are stored. The sv of *Pkd2* mutant males are of normal size compared to that of wild-type males of the same age (Figure 3A). *Pkd2* mutant sperm inside the sv are emptied normally during mating (Figure 3B), and a large amount of the mutant sperm are found in the female uterus immediately after mating (Figure 3H). The fertilization rate was measured by detecting the sperm tail inside the egg within 15–30 min after egg deposition. When females were mated with wild-type (*+; dj-GFP*) males, 79% of the resulting eggs contained a coiled sperm tail (n = 100) (Figure 3C), and the egg-hatching rate was 78.7% (n = 375). In contrast, only 3% of the eggs contained the sperm tail when eggs came from females mated with ($PKD2^{ko67}$; *dj-GFP*) mutant males (n = 200; data not shown), and the egg-hatching rate in this case was 2.7% (n = 295). This suggests that once inside the egg, $PKD2^{ko67}$ sperm are as fertile as wild-type sperm.

A wild-type *Drosophila* male ejaculates ~4000–6000 sperm in a single mating attempt. During mating, a gelatinous mating plug forms at the posterior uterus before sperm is transferred to prevent sperm leakage through the posterior opening [7]. Immediately following sperm deposition, wild-type sperm move away from the mating plug, and essentially all sperm congregate at the anterior uterus (Figures 3E–3G). At the anterior uterus, sperm avoid going into the large common oviduct (O) but instead pass through narrow openings that connect to the seminal receptacle (R) and spermathecae (S) (Figures 3D–3G), which are sperm storage organs where sperm are sorted and prepared for fertilization [8]. This series of directional sperm movements involving anterior congregation and translocation into the sperm storage organs is likely guided by directional cues (chemical or mechanical signals) in the female reproductive tract [8, 9]. Later on, separate signals allow the release of the stored sperm individually, which then enter the egg through the micropyle, a cone-shaped special sperm conduit on the eggshell.

The speed of wild-type sperm movement in the uterus is rapid. By the time copulation ends 10–12 min following sperm deposition, anterior sperm congregation has already completed and sperm storage organs appear full with sperm (Figure 3E). In contrast, an abnormal sperm

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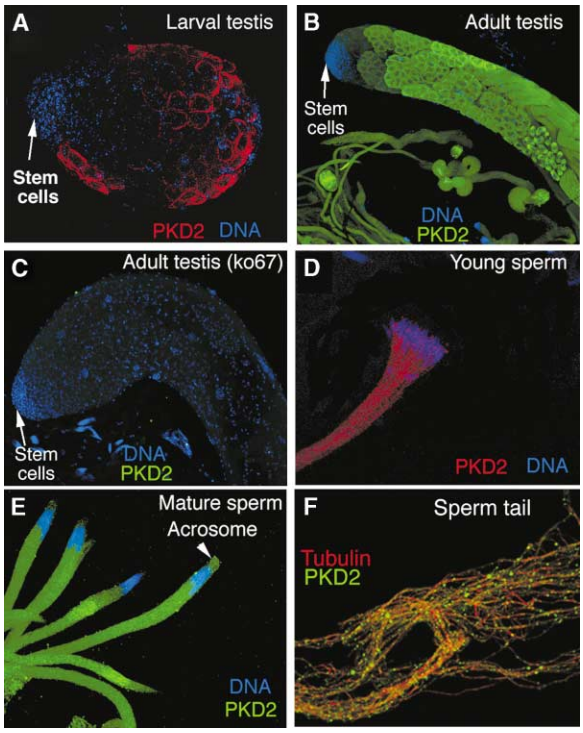

Figure 1. PKD2 Expression in Sperm Precursors and Sperm

In wild-type larval testis (A) and adult testis (B), all stages of differentiating sperm precursors except germline stem cells are stained positively with the anti-PKD2 antibody. In contrast, a *Pkd2*^{ko67} adult testis shows only background staining (C). Sperm develop from 64 spermatids as a syncycial bundle (D and E). The acrosome region, as indicated in (E), and sperm tail show particulate staining of PKD2. In individualized mature sperm tails (F) doubly labeled with tubulin (Red) and PKD2 (Green), PKD2 protein also appears as particulate staining.

movement pattern is observed in wild-type females mated with *Pkd2*^{ko67}*; DJ-GFP* males. The mutant male copulates for a similar length of time as the wild-type male, and the mating plug, which can be dissected out of the uterus as an autofluorescent plug [7], forms normally during mating (data not shown). The mutant sperm show similar but slightly less vigorous flagellar beating motion in dissected female uterus and spread away from the posterior ejaculation site to occupy nearly the entire uterus even before copulation ends (Figure 3H). However, *Pkd2* mutant sperm never show the anterior congregated pattern in the uterus as shown for the wild-type sperm, and none of the sperm storage organs are full even at 24 hr postmating (Figures 3H–3J; n = 80). The seminal receptacles dissected at 24 hr postmating are either completely empty (40%, n = 80, Figure 3K) or have 1–30 individual sperm (60%, n = 80, Figure 3L). Occasionally, mutant sperm are found stuck at the entrance to the receptacle (Figure 3K). Between 3–4 hr after mating, unstored mutant sperm leak out of the uterus more quickly than unstored wild-type sperm (Figure 3G versus 3J).

Previous studies show that mated *Drosophila* females exhibit avoidance behavior to subsequent mating attempts. Sperm storage in the female prolongs the avoidance behavior of the mated female for as long as 8 days, whereas females mated with spermless males will remate starting approximately on the second postmating day [9]. Wild-type females were first mated with *PKD2*^{ko67} mutant males and then challenged by wild-type males for remating. Accumulatively, 72.7% and 88.3% of these mated females (n = 82) remated on the second and third postmating day, respectively. The ratio of F1 progeny fathered by the first male versus the second male was 1/20 (n = 750), indicating a success of sperm competition by the second-mated wild-type male over the first-mated mutant male. In a parallel experiment, none of the wild-type females that were mated with wild-type males remated on the second postmating day, and only one out of 82 of these females remated on the third postmating day. These results confirm the sperm storage defect of *Pkd2* mutant sperm.

In summary, we observe that *Drosophila* sperm without a functional PKD2 channel show flagellar beating motion and movement in the female uterus but never congregate at the anterior uterus, and very few are found inside the sperm storage organs. The 3% of the eggs fertilized by the mutant sperm hatch at a normal rate, suggesting that rare mutant sperm that reach the egg are able to fertilize the egg and produce viable progeny. Sperm pass through the sperm storage organs before being utilized for fertilization in *Drosophila* [8]. *Drosophila* lay coated eggs that become activated by the ovulation process irrespective of fertilization by sperm [8]. Thus, in vitro experiments are probably not possible to directly test whether *Pkd2* also affects sperm-egg fusion as recently shown for a TRP family channel [10]. Our data suggest that *Pkd2* mutant sperm fail to respond to

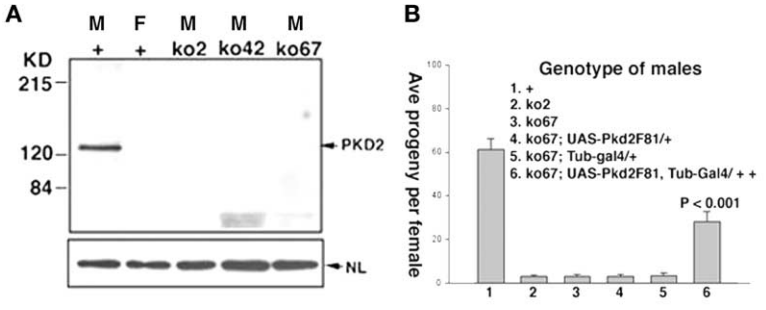

Figure 2. Western Blot and Male Fertility of *Pkd2* Alleles

In (A), sample orders are one wild-type adult male (M, +), one wild-type adult female (F, +), followed by one adult male of each *Pkd2* allele as shown. The blot was probed with anti-PKD2 antibody and anti-nuclear lamin (NL) antibody as loading control. Fertility was measured as the average numbers of progeny per mated wild-type female per 48 hr (y axis). The x axis (1–6) indicates the genotypes of the male parents tested (n = 20–28). The average numbers of progeny produced by males of each genotype are: 61.1(1), 2.85(2), 3.14(3), 3.07(4), 3.46(5), and 28.88(6). Note that both *UAS-Pkd2F81* and *Tubulin-Gal4* have to be present to produce a significant rescue of male fertility. Several independent transgenic insertions of *UAS-Pkd2* also rescued the fertility of *Pkd2*^{ko67} (data not shown).

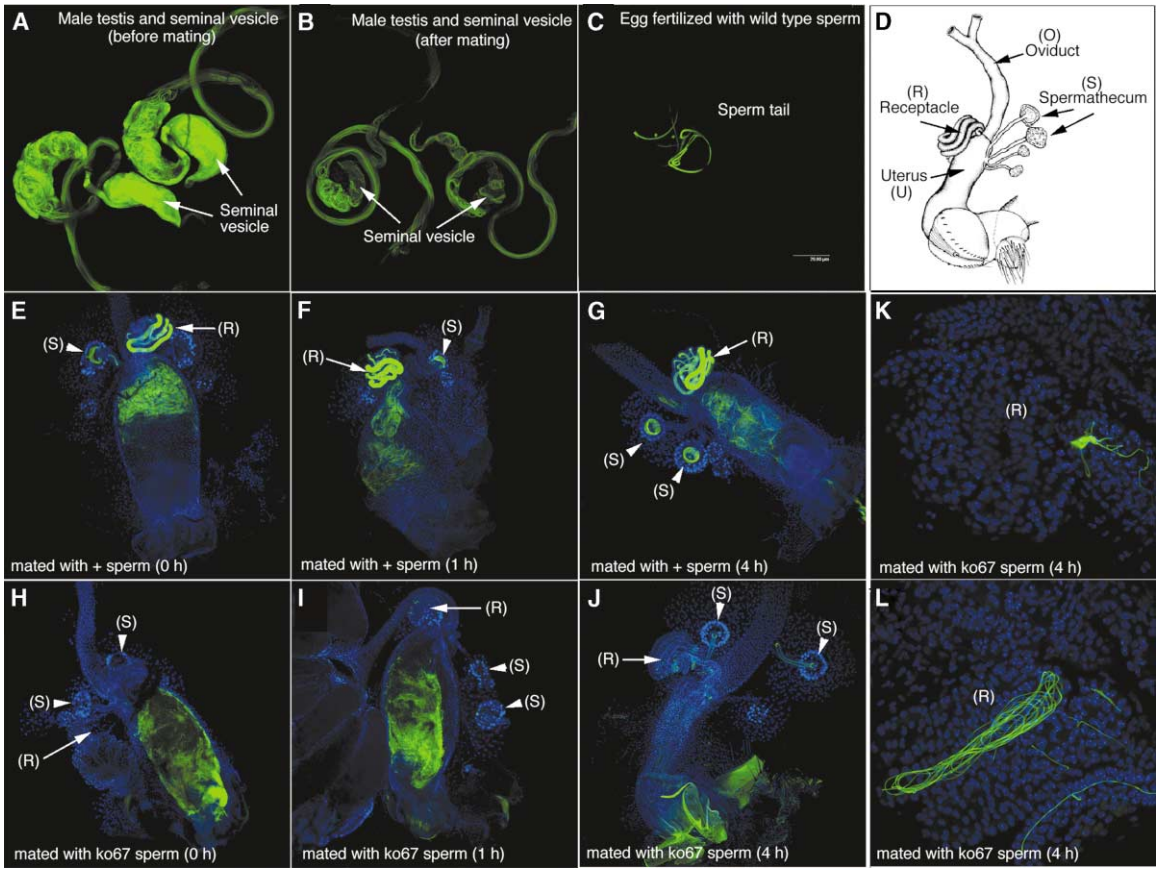

Figure 3. *Pkd2* Mutant Sperm Are Defective at Sperm Storage

In (A) and (B), the male reproductive organs of *Pkd2*^{ko67}*; dj-GFP* males (8 days old) without (A) and with (B) mating with females are shown, indicating that *Pkd2*^{ko67} sperm (marked green by *dj-GFP*) in seminal vesicles (arrows) are successfully released during mating. (C) shows a newly fertilized egg with coiled sperm tail inside. *Pkd2*^{ko67}*; dj-GFP* mutant sperm are able to fertilize eggs but do so at a greatly reduced rate of 3% compared to 79% for +*; dj-GFP* males. A schematic drawing of a female reproductive tract is shown (D). (E–L) Wild-type female reproductive tracts dissected at the hour indicated following mating with +*; dj-GFP* males (E–G) and *Pkd2*^{ko67}*; dj-GFP* males (H–L). (K) and (L) are high magnifications of receptacles (R) showing stuck sperm at the opening connecting to the receptacle tubes (K) and a few sperm inside the receptacle (L). For all panels, sperm tails are green, and nuclei of cells of the female reproductive tract are blue.

directional cues in the female reproductive tract. Candidates for the postulated directional cues are cell surface or secreted proteins derived from the female reproductive tract and/or ejaculated male seminal proteins. However, *Pkd2* is not expressed in the male accessory gland. The mating plug proteins and seminal protein Acp36ED, which plays a role in sperm storage [9], are properly transferred during mating (data not shown). Wild-type sperm are stored properly in *Pkd2* mutant females (data not shown), suggesting that *Pkd2* is only required in the male for fertilization. Therefore, *Drosophila* PKD2, a new member of the PKD2 family of Ca^{2+}-activated cation channels, is the first genetically defined component shown to function in sperm for directional movement inside the female reproductive tract.

In species that do not store sperm such as humans, sperm also travel directionally to meet the egg [11]. Interestingly, human sperm express a sperm-specific member of the PKD1 family, as do sea urchin sperm [12, 13]. There are increased incidences of male infertility in patients with ADPKD [14, 15]. The etiology was attributed to pituitary dysfunction in chronic renal failure or cystic changes in the seminal vesicles that led to obstructive azoospermia [14, 15]. ADPKD patients are heterozygous individuals and somatic inactivation of the wild-type copy is required for cyst formation [16]. Thus, if PKD2 has an intrinsic role in human sperm, it would probably be revealed in rare cases when dominant-negative mutations are involved. It is also possible that another PKD2 family member or none of the PKD2-like proteins functions in human sperm.

Many somatic cell types such as epithelia and neurons have single cilia. PKD1 and PKD2 proteins are localized on the cilia that have been shown to trigger calcium influx through PKD2 in response to mechanical or chemical signals involved in left-right body axis determination and inhibition of renal cyst formation [2, 4]. A sperm tail is a special type of cilium. The predominant localization of PKD2 in sperm tails is consistent with a role in directional movement that requires asymmetric flagellar beating or steering [11]. Since we show that *Drosophila* PKD2 protein exhibits Ca^{2+}-activated Ca^{2+}-permeable cation channel activity in the S2 expression system (C. Venglarik, Z.G., and X.L., unpublished data), it is conceivable

that PKD2 mediates asymmetric ion (such as calcium) influx at different points along the 1.9 mm long sperm flagellum, and this would probably provide the signal for initiating asymmetric flagellar beating and/or steering, leading to sperm congregation and storage at specific locations in the female reproductive tract.

Supplemental Data

Supplemental Data including experimental procedures and a figure are available at http://www.current-biology.com/cgi/content/full/13/24/2175/DC1/.

Acknowledgments

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Accession Numbers

The GenBank accession number for the *Drosophila Pkd2* cDNA is AY283154.